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ULTRASONIC VOCALIZATIONS OF INFANT SHORT-TAILED FIELD VOLES, *MICROTUS AGRESTIS*

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Ultrasonic vocalizations of infant rodents are used in developmental studies and for investigating the effects of drugs or environmental pollutants. Few studies, however, have analyzed the frequency characteristics of these ultrasonic vocalizations. This study investigates the physical and vocal development of infants (1–14 days old) of the short-tailed field vole, *Microtus agrestis*, under 2 conditions of isolation: at 23°C immediately after being isolated from the nest or at 23°C after 10 min of isolation at 27°C. Seventy-three percent of the infants vocalized, and there was great variation among calling infants in the number of vocalizations emitted. More infants, especially males, called during the 2nd period of isolation than during the 1st, and latency to call increased with age in males. The ultrasonic vocalizations were comparable to those recorded from North American voles. Vocalizations were classified into 7 categories on the basis of characteristics of the fundamental frequency. Simple calls were emitted most commonly and became more frequent in older infants, whereas the proportion of calls with a down-sweep in frequency and audible clicks decreased with age. The emission of ultrasonic calls, therefore, appears to reflect changes both in physical development of the infants and in their external environment.

Key words: development, field vole infants, isolation, *Microtus agrestis*, ultrasonic vocalizations

Ultrasonic vocalizations are produced by infants of a number of myomorph rodent species (Sales and Pye 1974; Smith and Sales 1980), but they apparently have not been detected from other rodents. They have been studied mainly in laboratory strains of rats (*Rattus norvegicus*) and mice (*Mus musculus domesticus*) and, to a lesser extent, in hamsters (*Mesocricetus auratus*) and gerbils (*Meriones unguiculatus*). The calls are used in developmental, psychological, and social studies and as indicators of the effect of drugs or environmental contaminants (Benton and Nastiti 1988; Di Giovanni et al. 1994; Holman et al. 1995). Calls of infants removed from the nest (isolated) are particularly useful in such studies because the calls generally occur from birth. Fewer studies have been made of wild rodent species, including voles (*Microtus*) and deer mice (*Peromyscus*). This study was part of a wide-ranging investigation into the development and use of ultrasonic calls by the British short-tailed field vole (*Microtus agrestis*—Mandelli 1998). This species is common in Britain and northern Europe and is used in laboratory studies of reproduction, parasitology, and stress-induced behavioral changes (Milligan 1974;

Redman et al. 1999; Soveri et al. 2000). The work reported here focuses on the development of ultrasonic vocalizations from birth to the beginning of weaning at 14 days. Blake's (1992) study appears to be the only other detailed study on infant vocalization in this species.

In *Microtus*, as in other myomorphs, the main situations and stimuli that elicit ultrasound emission by infants are isolation by removal from the nest together with changes in thermal conditions (Blake 1992; Colvin 1973; Noirot 1966; Okon 1970a; Rabon et al. 2001; Shapiro and Insel 1990), tactile stimuli (Colvin 1973; Okon 1970b), and olfactory stimuli (Kapusta et al. 1995). Ultrasound emission by infants appears to be related mainly to thermal vulnerability and the development of homeothermy. Most myomorph infants are heterothermic at birth but are resistant to cold stress; infants become less resistant as homeothermy develops (Lagerspetz 1962). Infant field voles removed from the nest vocalized more during their 2nd week after birth, when temperature maintenance was developing, than during either the 1st week, when body temperature was not maintained, or the 3rd week, when temperature was maintained at $\geq 36^{\circ}\text{C}$; they also called more at 5°C than at 20°C (Blake 1992). Ultrasound emission was therefore related to ambient temperature, although not to the infants' deep body temperature (Blake 1992). In North American species of *Microtus*, however, infant vocalization appears to be related more to degree of parental care and the mating system of the parents than to the

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development of thermoregulation. Infant prairie voles (*M. ochrogaster*) emitted more ultrasonic calls when removed from the nest than infant montane voles (*M. montanus*—Blake 2002; Shapiro and Insel 1990), although the development of thermoregulation is similar in both species (Blake 2002). Prairie voles are unusual among vole species in being monogamous and in having extensive biparental care; furthermore, the infants, which are seldom left alone in the nest, exhibit strong nipple attachment. Montane voles, like many other vole species, are polygynous. The female alone cares for the young, but she gives them less attention than prairie vole parents, and the young are less tenacious nipple clingers (Getz et al. 1993; McGuire and Novak 1984, 1986; Wilson 1982). The high rate of calling in prairie voles might be necessary to maintain their high levels of parental care (Blake 2002; Rabon et al. 2001).

There have been fewer studies of the frequency characteristics of infant vole calls than of general vocal behavior. The frequencies reported for infant *Microtus* calls vary somewhat with species and study. Reported frequencies are 40–55 kHz from 1-day-old infant woodland voles (*M. pinetorum*—Geyer 1979), a modal frequency of 35 kHz in 3-day-old common voles (*M. arvalis*—Motomura et al. 2002), and 25–60 kHz for field voles (Sales and Pye 1974). The field vole calls show slow downward drifts in frequency and sometimes step-like frequency changes. The duration of the calls of newborn infants is 40–120 ms, whereas in infants beginning to leave the nest, it is 3–30 ms. Colvin (1973) studied the frequency characteristics of several North American voles (California voles, *Microtus californicus*; meadow voles, *M. pennsylvanicus*; long-tailed voles, *M. longicaudus*; prairie voles, and montane voles). The ultrasonic vocalizations of infants aged 3–18 days when handled had a frequency range of 30–45 kHz, with a mean fundamental frequency of 32 kHz, bandwidths of 3–15 kHz, and durations of 60–150 ms (\bar{X} = 121 ms—Colvin 1973). For most of these species, the frequency of the fundamental component decreased with age, whereas in all except prairie voles, bandwidth increased with age. Prairie voles also differed from other species in tending to have a lower fundamental frequency. Colvin (1973) distinguished 4 types of call on the basis of the number of frequency components present and degree of frequency modulation: plain whistle (with continuous fundamental frequency and sometimes with harmonic components), whistle chirp (with a break in the fundamental and the 1st part longer than the 2nd), wavering whistle (with large enough fluctuations in frequency “to change the sound”—Colvin 1973:245), and hoarse whistle (apparently with 2 sections, as in whistle chirp, and with 2 or more frequency components above the fundamental that might or might not be harmonically related to the fundamental). Younger infants were observed to emit plain whistles more often than older infants, and older infants emitted more audible calls than younger infants (Colvin 1973).

In Blake’s study (1992) on the British field vole, the calls were not analyzed spectrographically, so they could not be compared with North American species. A further aim of the current work was, therefore, to compare as far as possible the calls of British field voles with the North American species

studied by Colvin (1973). Preliminary observations indicated that calling by infants was affected by the length of time out of the nest. In the above studies, the length of time between removing infants from the nest and the start of recording was not reported. The current study also investigated the effect of different periods of isolation (at ambient temperature) on emission of calls and, particularly, on their frequency characteristics. In addition, physical development of the infants was monitored, and an attempt was made to relate any changes in the vocalizations to developmental changes.

MATERIALS AND METHODS

Animals.—The infants studied were from at least 2nd-generation laboratory-bred parents and were born in the field vole colony at King’s College London. This colony was established more than 20 years ago from voles trapped at various locations in southeastern England (United Kingdom), and wild voles trapped in other locations in that area were introduced every 3–5 years to maintain genetic diversity. The colony was fed with complete rat pellets (PM1, Special Diet Service, Essex, United Kingdom), sunflower seeds, nuts, dried fruits, and fresh apples. Food and water were given *ad libitum*. The voles were housed in polypropylene cages ($13 \times 45 \times 12$ cm) and provided with sawdust and hay, and cages were cleaned twice per week. Room temperature was 23–24°C, and the light regime was 16L:8D, with lights off at 0900 h. The infants observed were 1–14 days old (day of birth = day 1). Older infants were not observed because initial observations showed that infants ≥ 15 days old would not call spontaneously when isolated (although infants are not naturally weaned until around 3 weeks of age—Gipps and Alibhai 1991).

A total of 259 infants (from 185 litters) was studied, with an average of 24 animals and a minimum of 15 animals for each day of age studied. From each litter, 1 or 2 infants (depending on litter size) were removed from the nest but never forcibly removed from nipples. Lactating females were always left with 1 infant. A maximum of 2 infants/litter was studied, 1 for each period of observation (see below). Where possible, 1 infant of each sex was taken and pairs were removed together. Infants were sexed by the distance between anal and genital openings (greater in males than in females) and the presence of conspicuous mammae in females. It was difficult to sex 1- and 2-day-old infants accurately, so results for these infants were pooled for analysis. For older infants, only those that could be sexed reliably were included in the study. Each infant was observed only once.

For the 1st period of observation, an infant was immediately placed on sawdust in a plastic box ($15 \times 21 \times 14$ cm) at room temperature (23°C) and observed singly for 10 min. Where possible, the sex of the infant observed 1st was alternated between successive tests. During this period of observation, the infant for the 2nd period was kept at 27°C on cotton wool in a glass jar under a white light to maintain body temperature above ambient levels and so reduce temperature-induced vocalizations before the observation began. The infant was protected by a sheet of paper to limit the effect of the white light. At the end of the first 10-min period, the infants were exchanged and the 2nd infant was observed for 10 min (2nd period of observation). The change of infants between the jar and the observation box took <1 min. At the end of both periods of observation, the 2 infants were put back in their nest and the sawdust in the test box was changed. The observations were carried out during the colony dark period, mostly between 1000 and 1400 h.

Physical development of all the infants was observed during the test sessions. The 1st indications of hair growth, development of mobility

(ability to crawl or walk in a coordinated way), opening of the eyes and ears, and eruption of teeth were monitored. The information given below is based on 50% of the infants studied attaining a particular level of development.

Ultrasound emission was detected by a QMC IRL S100 ultrasound detector (Ultrasound Advice, London, United Kingdom). The microphone was placed vertically 15 cm above the floor of the test box. The audio output of the detector was used to monitor ultrasound emission via headphones. The presence or absence of ultrasonic calls was scored on a check sheet over successive 5-s intervals by 1-0 sampling (Martin and Bateson 1993), giving a maximum score of 120 intervals. This sampling interval is considered to give a reasonable approximation of the rate of ultrasonic calling in rodents (Dizinho et al. 1978) and is sufficient to study the effect of different ages and different periods of observation on amount of vocalization emitted. Some infants also emitted broadband clicks that extended from the audible range to the ultrasonic range, and a few showed breaks in their ultrasonic vocalizations into the audible range; these sounds were also scored. The latency to ultrasonic calling was taken from the start of the observation to the start of the first 5-s interval during which at least 1 ultrasonic call was detected. When it was certain that an infant was vocalizing, the calls were tape recorded for 2 min continuously via the high-frequency output of the ultrasound detector onto one-quarter-inch (6.4-mm) magnetic tape at a speed of 30 inches/s (76 cm/s) with a Racal Store 4DS tape recorder. Young infants tended to call at the beginning of a 10-min session and older infants toward the end. The recordings were therefore made at different times in the session for different ages, but with fast tape speeds of 76 cm/s, it was not feasible to record for the whole of each observation period.

Sonographic analysis.—Recordings of infants emitting at a low rate or intensity were generally excluded from analysis because of insufficient numbers of vocalizations or poor quality of recording. Only where there was a minimum of 6 different, adequate recordings for each age and time period were statistical comparisons carried out. Some age and sex groups, therefore, had to be omitted from the comparisons. In this study, a single burst of ultrasound is termed a pulse, and a train of several pulses is termed a call. Between 10 and 30 pulses ($\bar{X} = 20$) were analyzed per infant. The recordings were replayed at one-eighth of the recording speed and analyzed on a Power Macintosh 7200/90 computer with Canary 1.2.1 software (Cornell Laboratory of Ornithology, Ithaca, New York). The sampling rate was 22,050 Hz and the sample size 16 bits. The narrowband filter setting was used to maximize frequency resolution. Ultrasonic calls of rodent infants generally show a gradual onset and offset, and recorded start and end times will depend on when call amplitude exceeds thresholds of the recording apparatus, on the position and orientation of the infant relative to the microphone, and on interference within the recording chamber. It was therefore felt that, given the large number of calls analyzed, the relatively small increased precision in measurement of temporal features by the use of a broadband filter was not justified.

Ultrasonic vocalizations were analyzed as individual pulses whether emitted in sequences or singly. They were chosen at regular intervals during the recording. When the pulses were emitted in sequences (calls), every other pulse was selected up to a maximum of 4. Six characteristics were measured: duration, initial frequency, terminal frequency, maximum frequency, minimum frequency, and bandwidth (maximum frequency minus minimum frequency). Often 1 or more harmonics could be observed, possibly due to input overload. The lowest frequency component, believed to be the fundamental, was used for measurements. The mean score per infant for each measure was used for statistical analysis.

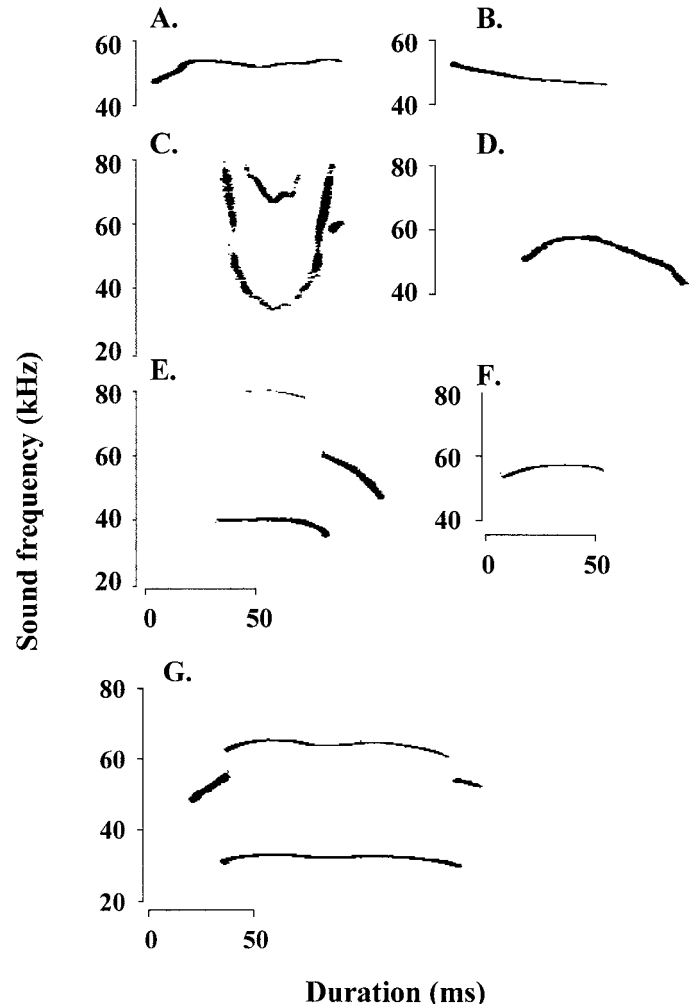


FIG. 1.—Representative sonograms of the 7 categories of pulses distinguished in isolated *Microtus agrestis* infants. A) Up-sweep pulses; B) down-sweep pulses; C) U-shaped pulses; D) n-shaped pulses; E) step pulses; F) simple pulses; and G) complex pulses.

The pulses were classified into 7 categories on the basis of a bandwidth >5 kHz or <5 kHz and on the direction of any frequency change. Although the various frequency patterns identified appeared to be part of a continuum, the pulses could readily be distinguished in this way; however, such a division is somewhat arbitrary (Fig. 1). Pulses with a bandwidth <5 kHz were classified as simple pulses. Those with a bandwidth >5 kHz were classified as to the direction(s) and nature of the frequency change. Up-sweep and down-sweep pulses showed a single direction of frequency change (upward and downward, respectively). The U-shaped and n-shaped pulses had 2 alternate directions of frequency change and had differences of >5 kHz between initial and terminal frequencies and either the maximum (n-shaped pulses) or minimum frequency (U-shaped pulses). Step pulses and complex pulses were pulses with 1–2 single-frequency jumps in the pulses or with several frequency breaks and modulations >5 kHz, respectively (Fig. 1).

Statistical analyses.—Results were analyzed to investigate effects of age, sex, and, within each sex, order of observation (i.e., 1st or 2nd period of observation) on number of infants calling, types of pulses emitted, and acoustic variables. Data are given as median (m) \pm q (interquartile range, which is the difference between the value of a data

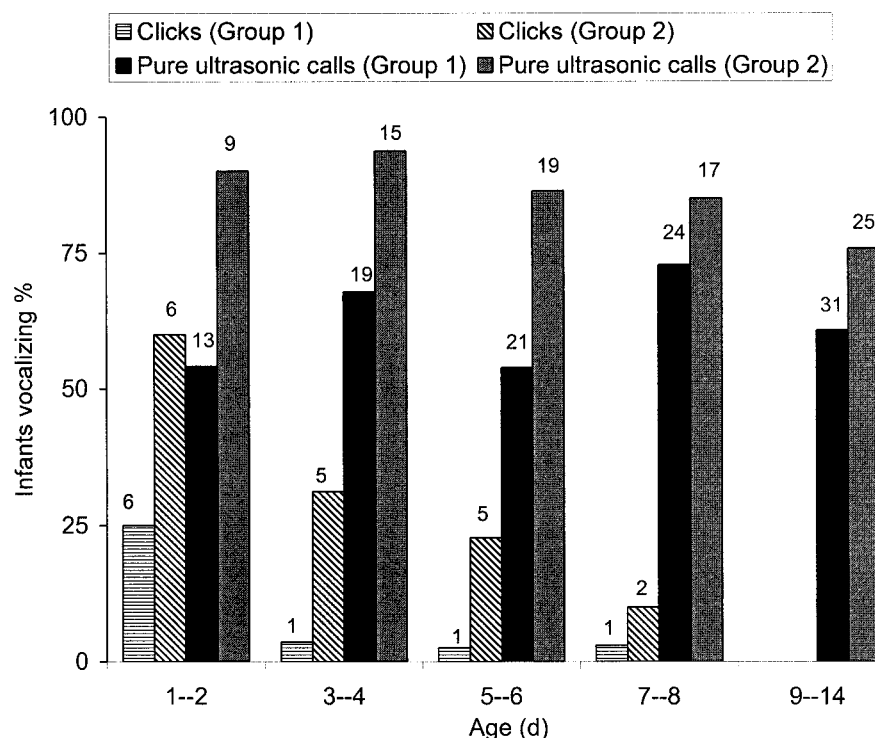


FIG. 2.—Emission of clicks and ultrasonic vocalizations: percentage of *Microtus agrestis* infants emitting clicks and pure ultrasonic pulses for each age group and period of observation. Number above each bar is number of infants calling: group 1—1st period of observation; group 2—2nd period of observation (after 10 min).

point 25% of the way down a ranked list and the value 75% down). Chi-square tests were used to compare the number of infants emitting clicks or pulses with breaks into the audible range. The number of 5-s intervals during which ultrasound was monitored (scored intervals), latency to call, and pulse characteristics were compared across age within each observation period for all infants, and for female and male infants separately, by Kruskal–Wallis 1-way analysis of variance (ANOVA). When this measure was significant ($P < 0.05$), a Bonferroni correction and multiple comparison test were carried out (Siegel and Castellan 1988). When percentages were compared (as in comparing the percentage of infants calling and of different categories of calls across age groups), an arcsine transformation was performed before statistical tests were applied. A GLM ANOVA test was then carried out by Minitab (Minitab Inc., State College, Pennsylvania—Ryan et al. 1985). In all comparisons, measures from the 1–2-day-old age group were used as a comparison to show changes with age from birth. Statistics are given only when they reached significance ($P < 0.05$) or when there appeared to be a meaningful trend.

RESULTS

No aggression was observed toward the infants when they were returned to the nest, and the test procedure was not observed to affect development of the test infants compared with other infants.

Physical development.—As in other infant voles, infants of *M. agrestis* were altricial at birth, with eyes closed, pinnae folded against the head, and pink skin. During the first 2 days after birth, the skin became darker; hair appeared at day 3. At about this age, the infants started to show coordinated movements and could crawl. Between 5 and 10 days of age,

locomotion began to develop, and by day 10, some infants could walk. Sensory development also occurred in successive stages. At day 2, the pinnae unfolded, but the meatal opening could not be seen easily, and a startle response to sudden sounds was observed only when the eyes were beginning to open from day 8 on. The eyes were totally open 10 d after birth in most cases. Teeth were 1st observed at day 6, but infants were observed to eat solid food only at around day 12. At this time, they left the nest spontaneously but were still suckling at day 20 and sometimes later.

Vocal behavior.—Of 259 infants observed, 189 (73%) emitted ultrasonic vocalizations and, apart from infants 13–14 days old, at least 50% called from each age, sex, and period of observation. However, due to the small number of infants vocalizing in each category on each day, results were pooled across consecutive days 1–2, 3–4, 5–6, 7–8, and 9–14. Not all tape recordings were suitable for analysis, so some age groups represented only males or females. Statistical analyses of the physical characteristics of the calls were therefore carried out across relevant age groups.

More infants emitted ultrasonic vocalizations during the 2nd period of observation (i.e., after 10 min of isolation) than during the 1st period (Fig. 2). Across all ages, a greater percentage of males called during the 2nd observation period ($F = 13.22$, $d.f. = 1, 9$, $P = 0.007$). More females called during the 2nd period, but the difference was not significant. Within each observation period, there were no significant differences between the various age groups of either sex in the number of infants calling or in numbers of infants of each sex calling.

The emission of broadband clicks occurred more during the 2nd observation period (Fig. 2). Clicks were mostly detected from the youngest infants (1–2 days old); no clicks were detected from infants >8 days old. When data on sexes were combined, the number of infants emitting clicks decreased significantly with age in each observation period (1st: $\chi^2 = 9.44$, $d.f. = 3$, $P < 0.05$; 2nd: $\chi^2 = 11.83$, $d.f. = 4$, $P < 0.05$). A few infants aged 3–4 days showed breaks in their ultrasonic vocalizations into the audible range, but only during the 2nd test period. When sexes were combined, the number of infants emitting audible calls during the 2nd observation period decreased with age ($\chi^2 = 12.9$, $d.f. = 4$, $P < 0.05$).

The number of 5-s intervals during which ultrasonic vocalizations were detected varied considerably between infants of the same age or sex from few pulses to pulses emitted in sequences during the entire observation. The median number of scored intervals rarely reached 70 of the 120 possible (Table 1). There was no effect of age or period of isolation on scores for males or females treated separately or when sexes were combined.

Infants showed individual variation in latency to call. Most called as soon as they were placed in the test situation. Males showed a significant increase in latency with age and for both observation periods (1st: Kruskal–Wallis $H = 15.93$, $d.f. = 4$, $P = 0.003$; 2nd: $H = 9.97$, $d.f. = 4$, $P = 0.041$). For both periods, $m = 0$ s and q was between 0 and 2.5 for all ages, except 9–14 days (at which time the latency increased slightly—1st: $m = 10$ s, $q = 15$; 2nd: $m = 5$ s, $q = 6.25$). For the 1st period, a multiple comparison test showed that males aged 9–14 days had a greater latency to call than other ages. For the 2nd period, no specific age differences were detected by a multiple comparison test, but the data show that latency tended to be greatest in the oldest infants.

Spectrographic analysis.—There was also great individual variation in all characteristics measured (Table 2). In general, the calls were at frequencies of 35–50 kHz with bandwidths of 5–10 kHz. Duration generally varied from 30 to 50 ms. In some recordings, sequences of up to 12 pulses occurred, with interpulse intervals of 150–250 ms. Sequences were separated by intervals of 400–2,600 ms. There were no significant differences between sexes, across ages within sex, or between observation periods. For males during the 1st period, there were nonsignificant trends toward a decrease in duration with age ($H = 4.64$, $d.f. = 2$, $P = 0.099$) and an increase in bandwidth to 3–4 days and decrease to 9–14 days of age ($H = 4.47$, $d.f. = 2$, $P = 0.107$; Table 2). During the 2nd period, there was a similar trend toward an increase in bandwidth for males 3–4 days old ($H = 4.74$, $d.f. = 2$, $P = 0.093$). No significant differences or trends were found for females.

There were significant differences in emission of pulse categories. For all ages and both sexes combined, simple pulses represented $m \approx 50\%$ of all pulses in the 1st observation period and 47% in the 2nd. Step pulses and up-sweep pulses represented about 6% and 8%, respectively, of pulses in the 1st period and 8% and 9%, respectively, in the 2nd period. Down-sweep pulses, U-shaped pulses, n-shaped pulses, and complex pulses were emitted rarely.

Proportions of different call types varied with age for some pulse types and in some situations, but there were no

TABLE 1.—Number of 5-s intervals (out of a maximum of 120 intervals) in which isolated *Microtus agrestis* infants vocalized, by age and sex. Period 1 is the 10-min period immediately after the infants were removed from the nest. Period 2 is the 10-min period beginning at least 10 min after infants had been out of the nest. No infants were observed in both periods. The amount of calling varies with age during both periods, but there is no statistical effect on calling of age or period of calling for either sex. Inter-quartile range is q .

Sex	Age (d)	Period 1		Period 2	
		Median	q	Median	q
Sexes combined	1–2	51	5.6	39.5	8.1
Females	3–4	67	9.6	46	8.8
	5–6	43.5	6.7	27.5	5.4
	7–8	28.5	7.5	7	4.5
	9–14	18	7.3	17	6.5
Males	3–4	13.5	2.4	68	7.4
	5–6	36	5.4	39	3.8
	7–8	16	3.3	41.5	6.3
	9–14	29	5.7	14	5.4

differences between observation periods (Tables 3a and 3b). Simple pulses became more common with age in both sexes and for both periods of observation, but the trend was not significant for the 2nd period of observation for males alone ($F = 3.25$, $d.f. = 2, 21$, $P = 0.06$) or for sexes combined ($F = 2.54$, $d.f. = 4, 46$, $P = 0.06$). Up-sweep pulses were emitted by both sexes, at all ages, and in both periods of study, and they showed significant but unsystematic changes with age in the 1st period with sexes combined ($F = 3.6$, $d.f. = 4, 46$, $P < 0.05$). Down-sweep pulses were only recorded from the youngest infants during the 1st period of observation, resulting in significant changes with age for both sexes (females: $F = 3.71$, $d.f. = 2, 26$, $P = 0.04$; males: $F = 4.17$, $d.f. = 2, 23$, $P = 0.03$). In the 2nd period, they were recorded from all ages of each sex, but no significant changes with age were detected. Males showed a trend to increase the emission of down-sweep pulses at 3–4 days of age ($F = 2.54$, $d.f. = 2, 21$, $P = 0.10$). U-shaped pulses were recorded only from the youngest infants during the 1st period of observation, but the change with age was not significant. In females, a nonsignificant decrease in these pulses was observed during the 1st period of observation ($F = 2.68$, $d.f. = 2, 26$, $P = 0.09$). No other pulse types showed any significant changes with age.

DISCUSSION

Our observations on physical development are similar to those of Blake (1992). The observation that infants start to eat solid food and leave the nest beginning at day 12 agrees with Blake (1992) and Gipps and Alibhai (1991), who report that infants can be weaned as young as day 14. The time of eye opening (days 7–10) and the occurrence of startle responses to sudden sounds from day 8 also agree with findings of Blake (1992); she also found that thermoregulation by infants improved from 3–4 to 11–12 days of age. Thermoregulation was not monitored in this study, but the appearance of body hair (observed here in 3–4-day-old infants) undoubtedly affects it.

TABLE 2.—Characteristics of ultrasonic pulses emitted by isolated *Microtus agrestis* infants at different ages and sexes. *n* is the number of recordings used for analysis. Some age groups were omitted because of insufficient suitable recordings (see “Methods”). Period and *q* as for Table 1. Despite some apparent changes with age, no significant changes with age or significant differences between the sexes were found for any call characteristics.

			Frequency (kHz)											
Sex	Age (d)	<i>n</i>	Duration (ms)		Initial		Terminal		Maximum		Minimum		Bandwidth	
			Median	<i>q</i>	Median	<i>q</i>	Median	<i>q</i>	Median	<i>q</i>	Median	<i>q</i>	Median	<i>q</i>
Period 1														
Sexes combined	1–2	12	37.22	33.79	46.02	4.39	46.25	7.56	52.05	8.75	40.45	13.05	10.77	11.92
Males	3–4	7	50.8	22.14	40.02	23.43	43.03	23.5	47.92	24.16	37.32	23.25	8.92	10.23
	9–14	6	31.45	23.05	41.60	7.02	41.56	7.67	42.61	9.19	37.68	8.12	5.87	4.15
Females	7–8	9	42.15	34.67	42.04	11.08	41.10	9.51	46.25	13.61	36.20	9.12	6.65	13.29
	9–14	6	34.16	3.25	48.35	10.28	46.95	11.78	51.41	9.72	42.38	11.36	5.01	5.2
Period 2														
Sexes combined	1–2	8	41	27.65	38.48	3.8	42.43	2.87	43.36	3.52	38.21	4.87	7.42	9.03
Males	3–4	9	41.62	21.83	43.33	14.83	41.59	12.22	50.12	21.63	41.41	13.12	9.93	10.38
	9–14	6	32.85	18.82	42.84	8.21	43.85	2.32	45.47	6.42	39.85	4.39	4.87	8.86
Females	5–6	7	40.61	20.58	37.13	13.98	44.2	18.39	48.03	20.93	35.18	10.92	4.03	11.21
	7–8	7	53.05	24.57	41.85	12.26	42.59	10.8	43.89	7.59	37.28	9.34	10.35	2.13

The ultrasonic calls analyzed here were similar to those reported for field voles and other *Microtus* species, including some North American species. The range of the fundamental frequency (35–50 kHz) and bandwidth (5–10 kHz) correspond with observations of Colvin (1973), Geyer (1979), Motomura et al. (2002), and Sales and Pye (1974). The relatively common simple pulses found in this study are similar to the pulses of meadow voles and California voles, described by Colvin (1973) as plain whistles or whistle chirps (in this study, pulses separated by more than 5 ms were distinguished as different pulses; therefore, a whistle chirp as defined by Colvin (1973) was defined here as 2 simple pulses). The hoarse and wavering whistles defined by Colvin (1973) were termed complex or step pulses by us and were rarely observed. *Microtus* infants from different species therefore appear to emit structurally similar types of pulses. Further comparative and anatomical studies might indicate whether the types of ultrasonic vocalizations are related to physiological or anatomical characteristics. More detailed studies of the various types of calls and their prevalence in a range of species are needed to determine how far phylogenetic relationships might be reflected in physical characteristics of the calls.

The proportion of infants observed vocalizing by us (73%) was similar to that in other studies (e.g., 60–100% of infant Norway rats aged 3–15 days vocalized at 22°C—Oswalt and Meier 1975). Variable rates of vocalization also are widely reported as in our study (Blake 1992; Colvin 1973). No sexual differences were found in any of the measures studied, so this is unlikely to be a cause of interindividual variation. Sexual dimorphism in calling has been reported in infant Norway rats by Naito and Tonoue (1987) but was not found by Oswalt and Meier (1975). In this study, the infants that failed to vocalize might have been habituated to handling, although handling time during cage cleaning was minimal and handling was gentle. The interval since the infants had fed could also have influenced calling.

No significant changes with age were found in the amount of vocalization, contrary to previous studies on voles and other species, in which the rate of ultrasonic vocalization changed significantly with age and was related to the development of homeothermy (Blake 1992; Noirot 1966; Noirot and Pye 1969; Okon 1970a, 1970b). Blake (1992) counted the number of calls emitted by infant field voles over 20 min and found a peak of ultrasonic calling at age 10 d. The difference between her study and ours could be due to the time division of 5 s in this study for monitoring the ultrasonic vocalizations rather than total number of calls; to the shorter period of isolation from the nest used here; or to the relatively low numbers of infants calling in each age and sex group. It seems unlikely that the acoustic behavior of field voles differs from other infant myomorphs, so this aspect of their behavior deserves further study. There was some change with age in latency to call which, in both sexes, increased in infants of 9–14 days old. At this age, infants have improved thermoregulatory abilities, which might explain the increased latency to call.

Significant changes with age occurred in the emission of certain pulses during both periods of observation. Clicks and breaks from ultrasonic to audible calls were found only in young infants and could reflect the immaturity of the vocal mechanism. In this study, simple pulses became more common as the infants grew older, but Colvin (1973) found that plain whistles became less common relative to audible calls.

Some changes with age occurred in the prevalence of up-sweep calls in this study, but the pattern was complex and difficult to interpret. The changes that occur in ultrasonic calling might reflect physical changes that are occurring at this time in, for example, respiration, mobility, or vocal anatomy.

The period of observation had an effect on proportion of infants calling, particularly for males, and on the production of clicks and ultrasonic calls with audible components. This means that slight differences in protocol between studies could have effects on the reported acoustic behavior of infants. In

TABLE 3.—Categories of pulses emitted by *Microtus agrestis* infants during 2 successive periods of isolation as a function of age (1 infant/litter for each period of isolation was observed). Median and *q* values correspond to the percentages of the different types of calls emitted during 1 period of observation. *n* is the number of recordings used for analysis. Some age groups were omitted because of insufficient suitable recordings (see “Methods”). Simple calls were most common; down-sweep, n- and U-shaped, and complex pulses were rarely emitted.

Pulse type		Period 1			Period 2		
Sex	Age (d)	<i>n</i>	Median	<i>q</i>	<i>n</i>	Median	<i>q</i>
Simple pulse							
Sexes combined	1–2	12	43.35	17.27	8	45.00	58.50
Females	5–6				7	61.50	41.22
	7–8	9	66.70	45.00	7	40.00	9.30
	9–14	6	69.30	26.28			
Males	3–4	7	50.00	29.10	9	45.40	27.90
	9–14	6	56.20	52.60	6	73.35	24.97
Up-sweep pulse							
Sexes combined	1–2	12	4.50	15.15	8	10.00	45.62
Females	5–6				7	8.65	3.77
	7–8	9	0.00	9.00	7	9.00	14.10
	9–14	6	21.80	34.05			
Males	3–4	7	18.70	13.30	9	9.00	16.00
	9–14	6	7.70	9.15	6	6.65	21.65
Down-sweep pulse							
Sexes combined	1–2	12	7.10	11.35	8	0.00	4.50
Females	5–6				7	3.10	8.30
	7–8	9	0.00	0.00	7	0.00	4.50
	9–14	6	0.00	0.00			
Males	3–4	7	0.00	0.00	9	9.00	18.00
	9–14	6	0.00	0.00	6	00.00	1.67
n-Shaped pulse							
Sexes combined	1–2	12	0.00	0.00	8	0.00	0.00
Females	5–6				7	0.00	0.00
	7–8	9	0.00	0.00	7	00.00	0.00
	9–14	6	0.00	0.00			
Males	3–4	7	0.00	0.00	9	0.00	0.00
	9–14	6	0.00	0.00	6	0.00	0.00
U-shaped pulse							
Sexes combined	1–2	12	0.00	6.27	8	0.00	0.00
Females	5–6				7	0.00	6.22
	7–8	9	0.00	0.00	7	0.00	5.00
	9–14	6	0.00	0.00			
Males	3–4	7	0.00	0.00	9	0.00	10.00
	9–14	6	0.00	0.00	6	0.00	0.00
Step pulse							
Sexes combined	1–2	12	8.25	22.75	8	0.00	18.50
Females	5–6				7	13.35	16.67
	7–8	9	0.00	9.00	7	10.00	8.60
	9–14	6	31.25	7.78			
Males	3–4	7	0.00	25.00	9	10.00	12.20
	9–14	6	15.40	25.00	6	3.35	6.70
Complex pulse							
Sexes combined	1–2	12	0.00	19.27	8	3.50	10.62
Females	5–6				7	0.00	0.00
	7–8	9	9.50	2.00	7	27.00	18.90
	9–14	6	0.00	4.68			
Males	3–4	7	12.50	20.00	9	0.00	9.00
	9–14	6	6.70	25.00	6	6.70	5.00

this study, different stimuli were involved for the 2 periods of observation, and it is not clear whether it was the reduced temperature, increased handling, changes in tactile stimuli from cotton to sawdust, or changes in chemical stimuli that affected ultrasonic behavior of infants in the 2nd observation period. Effects of differences in protocol on calling make it difficult to compare studies, and such variables should therefore be controlled and reported. This study has shown that infant field voles, like other vole species, emit ultrasonic calls that can vary in a number of different characteristics. There are some changes in ultrasonic calling with age, but more studies are needed to determine fully the nature and extent of these changes and their relation to physical development. It has also been shown that differences during the period of isolation before calls are recorded can have marked effects on ultrasonic behavior.

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